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# Lygus Bugs and Seed Quality in Lentils (Lens culinaris Medik.)

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## ABSTRACT

Physical deformations, referred to colloquially as "chalky spot," are not uncommon on lentil (*Lens culinaris* Medik.) seeds harvested from crops grown in eastern Washington and northern Idaho. Seed lots with chalky spot command prices inferior to undamaged ones, but farmers have not been able to pinpoint how or when the damage occurs. Here we summarize the opinions of local producers; describe a number of varied investigations on damaged and undamaged seeds; and report the consequences when *Lygus* bugs are caged onto pot-grown plants in a glasshouse.

Data from each of these diverse types of investigations lend mutually supporting evidence to the hypothesis: Chalky spot syndrome of lentil seeds is the result of *Lygus* bugs feeding on immature reproductive structures, which probably results in the formation of poor quality seeds and reduced economic yield. Caging bugs onto pot-grown plants at the appearance of first flowers increased the subsequent prevalence of shrivelled, unfilled pods by 13.0 to 15.1 percent (three cultivars); promoted the abscission of immature pods by 22.6 percent on cv. 'Chilean' (the cultivar that dominates commercial production in the Palouse), but did not significantly affect pod abscission on two exotic accessions; and increased seed abortion by 4.3 to 19.7 percent (three cultivars), thereby reducing yields by 18.1 to 26.2 percent compared with *Lygus*-free control plants. Then again, between 16.4 and 40.2 percent of the seeds harvested had chalky spot damage, whereas none were damaged on plants kept free from *Lygus* throughout growth.

Scanning and transmission electron micrographs of damaged and healthy cotyledons dramatically exposed the consequences of chalky spot syndrome for cellular integrity. Damaged cells contained very little rough endoplasmic reticulum, but osmiophilic droplets, never seen in healthy cells, were common. These droplets may have been "congealed" cellular contents, "coagulated" membranes, or extra-cellular in origin. Damaged cells seldom contained the starch grains common in undamaged ones.

Cooked samples comprising 10 or 20 percent chalky spot seeds (by weight) were evaluated for sensory characteristics, texture, and color. Samples with damaged seeds were slightly, but not significantly, "less sweet," "more bland," and "more prone to become mushy during cooking." These changes, although undesirable, could not, by themselves, be the basis for downgrading seed lots comprising up to 20 percent chalky spot seeds by weight.

The consequences of chalky spot may persist into storage and through to subsequent crops. Damaged seeds seem prone to more rapid deterioration in storage. They leaked cellular contents more readily when imbibed, and they were more susceptible to attack from fungal pathogens; some (about 5 percent) did not contain embryos. Germination of damaged seeds was retarded and less successful

than in visibly healthy seeds; the seeds that did germinate often produced abnormal seedlings with morphological characteristics similar to those associated with mechanical damage in other grain legumes.

The priorities and objectives of subsequent research addressed to this problem are discussed.

**KEYWORDS:** *Lentils, Lens culinaris, Lygus hesperus, Lygus elisus*  
(Heteroptera, Miridae), seed quality, chalky spot

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# LYGUS BUGS AND SEED QUALITY IN LENTILS (LENS CULINARIS MEDIK.)<sup>1</sup>

By R. J. Summerfield, F. J. Muehlbauer, and R. W. Short<sup>2</sup>

## INTRODUCTION

Infestations of *Lygus* spp., notably *L. hesperus* Knight, *L. elisus* Van duzee, and *L. lineolaris* (Palisot de Beauvois) in Europe and North America in general and in the Pacific Northwest in particular (Shull 1933; Landis and Fox 1972; Hagel 1978),<sup>3</sup> can be especially destructive to crops of seed alfalfa, snap and lima beans, cowpeas (blackeye peas), soybeans, peas, birdsfoot trefoil, cotton, sugarbeets, carrots, tomatoes, safflower, guayule, magnolia, apples, strawberries, peaches, nursery stock, and other crops of local importance (Gupta et al. 1980). From the diverse spectrum of insect pests that commonly attack grain legume crops at all successive stages of development, *Lygus* spp. are regarded primarily as pests of meristematic tissues (for example, stem apices) and, especially, of young reproductive structures (Strong 1970; van Emden 1980). Irrespective of the crop infested and the species of *Lygus* responsible, typical depredations include localized wilting, necrosis, and malformation of tissues; abnormal morphology because of damage to stem apices; abscission of reproductive structures (notably unopened buds, open flowers, and immature fruits); and deformations of fruits and/or seeds (for example, Mehary 1976; Gupta et al. 1980). Infested crops may suffer a loss of economic yield, or a loss in product quality, or both.

With grain legume crops grown for seed, where yields are already relatively small compared with many nonlegume crops and notoriously variable between locations and in different years (Summerfield 1980a), losses in the quantity or quality of green or ripe seeds can be especially important. Most research on seed quality has focused on lima beans (*Phaseolus lunatus* L.), snap beans (*P. vulgaris* L.), and blackeye peas (*Vigna unguiculata* (L.) Walp). In all three

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<sup>3</sup>The year in *italic*, when it follows the author's name, refers to Literature Cited, p. 37.



species, the consequences of *Lygus* infestation are similar: damaged seeds of all sizes are misshapen, pitted, or discolored and may or may not have cracked testas; other seeds shrivel completely (for example, Baker et al. 1946; Elmore 1955). The damage has been variously described as "chalky spot," "yeast spot," "pitting," "sting spot," and "puncturing" (for example, Wingard 1922; Shull and Wakeland 1931).

Some researchers have likened *Lygus* damage on grain legume crops in general to stink bug damage in soybeans (Daugherty et al. 1964; Miner 1966). Whether challenged by *Lygus* spp., or by *Euschistus servus* (Say), or *Acrosternum hilare* (Say), the brown and green stink bug, respectively, a consensus seems to be that piercing, sucking mouth parts cause localized mechanical damage to a number of cells, which is then compounded by a combination of the consequences of salivary secretions and/or removal of cell contents (for example, Gupta et al. 1980) and by secondary microbial infections. Immature seeds seem more prone to damage than older ones--perhaps because ontogenetic changes in the physical properties and texture of integument tissues (as in peas; Reeve 1946) render seeds progressively more resistant to stylet penetration (and see Shull 1933).

We are not aware of any reports that indicate that *Lygus* spp. are a major, or indeed, a minor pest of lentil (*Lens culinaris* Medik.) crops, either in the United States or elsewhere (Manara et al. 1977; Kabir 1978; Singh et al. 1978; Duke 1980; Cubero 1981). Pod-borers, aphids, weevils, and cutworms usually represent the most important pest constraints to production (for example, Singh and Dhooria 1971; Kooner et al. 1978; Hawtin and Chancellor 1979; Hawtin et al. 1980). Nevertheless, physical deformations are not uncommon on lentil seeds harvested from crops in eastern Washington and northern Idaho. In appearance, the blemishes appeared to us remarkably similar to those resulting from *Lygus* infestations on other grain legumes. Farmers have adopted the colloquialism "chalky spot" to describe the deformed seeds and are increasingly concerned about their loss in crop revenue. Seed lots with chalky spot are downgraded and command a price inferior to lots without blemishes.

In this publication, we summarize the various opinions of local producers as to the probable cause of chalky spot in lentils; describe a number of varied investigations of the attributes of damaged and undamaged seeds; and report the consequences when *Lygus* bugs were caged onto pot-grown plants in a glasshouse. Our objective was to seek mutually supporting evidence to support or refute the hypothesis: Chalky spot syndrome of lentil seeds is the result of *Lygus* bugs feeding on immature reproductive structures.

## MATERIALS AND METHODS

### Magnitude of the Problem, Farmers' Opinions, and Pointers for Research

We have solicited information from the Washington and Idaho Dry Pea and Lentil Commissions, and from local producers of lentils, and summarize below their collective experiences during the last 5 years. Since the rolling Palouse hills of eastern Washington and northern Idaho produce almost all of the U.S. lentil crop, and the United States is the largest exporter of the crop (Summer-



field et al. 1982), then this information is relevant not only locally but also nationally and internationally.

## Physical, Chemical, and Physiological Characteristics of Damaged and Undamaged Seed Lots

Batches of 1,000 seeds of each of damaged (chalky spot) and undamaged lots were selected at random from each of four accessions from the USDA germplasm collection obtained from breeders' plots at a single location (Genesee, Idaho) in the same cropping season (1980). Accession LC711981 (soon to be registered and released as cv. 'Brewer') has yellow cotyledons and a proven ability to outyield cv. 'Chilean', which currently dominates commercial production in the Palouse; accessions VW000457, VW000563, and 'Redchief' are all smaller-seeded, red cotyledon types with large yield potentials.

### Interseed and Intraseed Dry Matter Distribution

To quantify the effects of chalky spot not only on average seed dry weight but also on the distribution of seeds relatively larger or smaller in size, every seed was weighed individually on a digital readout, top-pan balance and allocated to an appropriate weight class. Successive classes from the smallest to the largest had a maximum weight range of 5 mg.

The water contents (percent fresh weight) of five replicate batches, each of 20 damaged and undamaged seeds, were determined by appropriate weighings before and after drying the seeds 72 h to constant weight in a forced draught oven at  $80 \pm 1^\circ\text{C}$ . Similar sample sizes and numbers of replicates were also used for estimations of the relative contributions of cotyledons and testa to the dry weight of whole seeds. Since embryos represent slightly less than 2 percent of the total dry weight of mature lentil seeds (Singh et al. 1968), we did not attempt to dissect them out for weighing separately from the cotyledons. Only seeds from the weight class 51 to 55 mg were used; they were imbibed for 24 h at  $25^\circ\text{C}$  to facilitate removal of the testa, and both components were then dried to constant weight as described above.

### Germinability

Germinability of damaged and undamaged seeds from the respective median class weight range of each cultivar was estimated according to Association of Official Seed Analysts (1978) regulations. Five replicates each of 30 seeds were incubated at  $20^\circ\text{C}$  and 100 percent relative humidity in wet paper towels in the dark, and germination counts (emergence of a radicle at least 2 mm long) were made after 5 and 10 days. In addition, triplicate batches, each of 10 seeds, of all four cultivars were imbibed for 24 h at  $25^\circ\text{C}$ , and their cotyledons were then separated to see whether embryos were present.

## Chemical Composition

Batches of damaged and undamaged seeds, comprising only those seeds weighing between 41 and 45 mg, were homogenized into fine powder using a Cyclone Sample mill. Seeds of all four cultivars within this weight range were combined before grinding, and four subsamples, each weighing about 5 to 6 g, were digested in a mixture of concentrated nitric and perchloric acids and analyzed for P, K, S, Fe, Mg, and Ca content by inductively coupled plasma-atomic emission spectrometry (Benton Jones 1977; Dahlquist and Knoll 1978).<sup>4</sup> The total protein-N content of each subsample was estimated by Kjeldahl digestion according to standard laboratory procedures.<sup>5</sup>

## Estimates of Seed Vigor

Some insight into the physiological status of seeds can be gained from measurements of the electrical conductivity of steep water in which known weights of seeds have been soaked for a given period of time in a controlled temperature regime (Copeland 1976). This test is based on the premise that as seeds age and deteriorate, cellular membranes become more permeable and cell contents, especially sugars, are more likely to leak out into a bathing solution. Not surprisingly, mechanical damage greatly increases the rate of leakage and conductivity values are correspondingly larger (Copeland 1976).

In general, for any given species, the larger the conductivity the poorer the seed lot and the less likely seeds are to germinate in the field (for example, Maguire et al. 1973; Mathews and Bradnock 1967). Then again, seeds damaged mechanically are more susceptible to invasion by micro-organisms and, even if they do germinate, are more prone than undamaged seeds to produce morphologically abnormal seedlings (Baker 1972). While we recognize that the conductivity test is far from an absolutely reliable indicator of seed vigor (Copeland 1976; and see the cogent discussions by Roberts and Ellis 1980), and that mechanical damage is not strictly comparable to chalky spot syndrome, estimates of the "tendency to produce abnormal seedlings" and of the "relative leakability" of damaged compared with visibly healthy lentil seeds are likely to be useful indicators of gross differences in seed quality.

Four replicates each of 50 seeds of the same four accessions as used previously, and damaged or not by chalky spot syndrome, were weighed and then soaked in 250 ml water in separate conical flasks for 24 h at 20°C. The pH and electrical conductivity of water in each flask was measured at the outset and again after the 24 h had elapsed. Control flasks, without seeds, were included for each accession. Seeds from the same stocks were surface sterilized according to standard procedures (Summerfield 1980b) and sown 10 to each 15-cm-

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<sup>4</sup>Plant and Soil Analytical Laboratory, Department of Plant and Soil Sciences, University of Idaho, Moscow, Idaho 83843.

<sup>5</sup>Same as footnote 4.

diameter polyethylene pot in a glasshouse. Full details of plant husbandry and culture, and of environmental regulation, are presented below (see "Caging *Lygus* Bugs Onto Pot-Grown-Plants"). Ten pots per cultivar received only visibly healthy seeds and 10 others, only seeds damaged by chalky spot. The rates and success of emergence and the incidence of abnormal seedlings were recorded.

## Microscopy

Previous research on Leguminous and other plant species has shown that *Lygus* bugs, as they feed, can damage small groups of adjacent cells: cell walls, cellular contents, and organelles can be disrupted physically and lysed (disintegrated) chemically (for example, Flemion et al. 1954). Presumably, stylet penetrations will also leave holes (which may or may not become occluded by materials leaking from damaged cells and/or secondary fungal infestations) in the tissue challenged. Furthermore, insect mouth parts may be broken accidentally and left behind after feeding. Thus, if chalky spot syndrome of lentils is caused by feeding of *Lygus* bugs (or any other insect with similar mouth parts and feeding habits) then analyses of the internal structure of damaged seeds might be expected to reveal one or more symptoms in keeping with this type of degradation, namely: damaged, disrupted, or fragmented cell walls and cellular membranes; abnormal configurations of starch granules and other macromolecules; fragments of insect mouth parts; and evidence of puncturing of outer cells in keeping with the diameter of stylets. Scanning and transmission electron microscopy techniques were used in an attempt to detect such abnormalities in damaged tissues.

## Scanning Electron Microscopy

Fruits at various stages of development were collected at random from the plants caged with *Lygus* bugs and separated into pod walls and seeds. The seeds were soaked in aerated water for 4 h at room temperature and then fixed for 3 days (again at room temperature) in a solution of 2 percent (para-) formaldehyde and 2.5 percent glutaraldehyde in 0.05M sodium cacodylate buffer at pH 7.0 (Karnowsky 1965), or with 3 percent glutaraldehyde in 0.1M sodium cacodylate buffer at the same pH. Both pod walls and seeds were dissected while immersed in these solutions to facilitate penetration of the respective fixative. Specimens were rinsed briefly in either 0.05M or 0.1M sodium cacodylate buffer at pH 7.1, postfixed for 2.5 h with 2 percent osmium tetroxide in distilled water, and dehydrated through a graded ethanol series.

Selected specimens were also subjected to ethanol cryofractography to reveal intracellular detail (Spurlock and Johnson 1974). A continuous fluid exchange device (Cohen 1974) was used to transfer specimens from absolute ethanol to Freon TF; they were then dried to the critical point (nearly all moisture removed) in a Bomar SPC-50 EX apparatus using Freon TF as the intermediate fluid, and CO<sub>2</sub> as the transitional fluid. The samples were given a gold coating, 300Å in thickness, in a Technics-Hummer sputtering device, and observed using an ETECA Autoscan U1 scanning electron microscope operated at 20 KeV. Adult *Lygus* bugs, collected from the same plants and at the same time as fruits, were fixed in 70 percent ethanol, dehydrated, and processed exactly as described above.

## Transmission Electron Microscopy

Pod walls and seeds, collected as described above, were fixed for 8 h with the formaldehyde-glutaraldehyde solution and postfixed for 1.5 h with 2 percent osmium tetroxide in 0.1M sodium cacodylate buffer at pH 7.0. Specimens were dehydrated through a graded ethanol series, vacuum infiltrated for 7 h at room temperature in either Spurr's (Spurr 1969) or Epon 812 epoxy resin and then embedded in the respective fresh resin. Silver sections, cut with a Sorvall MT2-B ultramicrotome (with either glass or diamond knives), were mounted on Formvar coated grids, stained with uranyl acetate and lead citrate (Reynolds 1963), and examined with an Hitachi H-300 transmission electron microscope operated at 75 kiloelectronvolts.

### Caging Lygus Bugs Onto Pot-Grown Plants

Replicate plants of three lentil genotypes of diverse origin and habit (table 1) were grown in a glasshouse in which day and night temperatures, but not daylength, were controlled.

Table 1.--*Lentil accessions exposed to Lygus bug infestation in a glasshouse*

Accession	Comments	Reference
Chilean '78	Yellow cotyledons; partially selected and refined land race; widely cultivated in the Palouse	Washington State Crop Improvement Association 1981
Precoz	Yellow cotyledons; successful in Argentina	Riva 1975
LC800028 <sup>1</sup>	Red cotyledons; very small seeds; originally from India	From ICARDA <sup>2</sup>

<sup>1</sup>USDA germplasm accession number.

<sup>2</sup>Accession No. ILL 4378 from the International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

Plants were grown in basally perforated, 15-cm-diameter (0.75-L capacity) polyethylene pots. A layer of crushed gravel (3 cm deep) was put in the bottom of each container to facilitate drainage, and all pots were filled to within 5 cm of the top with a medium of field soil, peat, coarse sand, and crushed gravel mixed in the ratio of 1:1:1:0.2 by volume, respectively. After sowing three



seeds into each pot, emergent seedlings were graded for uniformity and thinned to leave single plants. The area available to each plant was comparable to that in field crops seeded at reasonable densities (a seeding rate of  $67 \text{ kg ha}^{-1}$  with  $15,400 \text{ seeds kg}^{-1}$  to give  $1 \times 10^6 \text{ plants ha}^{-1}$ ; Muehlbauer 1973). Other aspects of plant culture and husbandry have been described in detail elsewhere (Summerfield and Muehlbauer 1982a).

Natural daylengths varied between 9 h 45 min and 12 h 40 min during a 68-day "vegetative" period from 22 January (sowing) to 31 March. Day and night temperatures were  $22 \pm 4.9^\circ$  and  $12 \pm 1.3^\circ\text{C}$ , respectively, which approximated to average values of meteorological screen daytime maxima ( $21^\circ\text{C}$ ) and nighttime minima ( $7^\circ$ ) temperatures during the first 60 days of lentil crop duration in a warm spring in the Palouse region of eastern Washington and northern Idaho (Summerfield and Muehlbauer 1982b). Mean maximum and minimum relative humidity varied between  $70 \pm 17$  and  $36 \pm 10$  percent. Banks of Sylvania VHO cool white fluorescent tubes, positioned 90 cm above pot level, maintained irradiance for 8 h each day (0800 h until 1600 h) at values ranging between 60 and 103 watts per meter square (400 to 700 nm; with complete cloud cover and cloudless, respectively), equivalent to a mean luminous energy of  $1750 \pm 59$  and  $3092 \pm 374 \text{ fc}$ , respectively.

After 68 days from sowing, when at least some replicates of all three cultivars had initiated flowers (in cv. Chilean, the first flower had opened (corolla color visible) whereas in cv. 'Precoz' and accession LC800028 several flowers had opened fully), 15 replicates of each cultivar were placed into large insect-proof cages. Two cages with wooden frames (1.02 by 1.53 by 1.08 m; to give a floor area of  $1.56 \text{ m}^2$ ) and nylon mesh ( $0.5 \text{ mm}^2$  open area) were used. Adults of *Lygus hesperus* and *L. elisus* (about 90 and 10 percent, respectively) were introduced into one cage to create an original infestation of 0.63 bugs per plant, which, based on experience with other hosts, might reasonably be expected to be injurious if, indeed, lentils prove susceptible to the pest (R. E. Fye, personal communication, 1981). Plants in the control cage remained insect-free throughout. Plants in both cages experienced a 16-h photoperiod with mean maximum and minimum temperatures and relative humidities maintained close to  $29^\circ$  and  $17^\circ\text{C}$ , and 49 and 32 percent, respectively.

All plants were allowed to fruit, mature, senesce, and die. Pods were then collected and counted, and records were taken of the numbers obviously misshapen or malformed and of the number and weight of damaged and undamaged seeds.

### Chalky Spot Damage, Cookability, and Taste

Seeds of those pulses (grain legumes) traditionally processed for human consumption in the United States, such as *Phaseolus vulgaris* (beans), *Phaseolus lunatus* (lima beans), and *Vigna unguiculata* (blackeye beans or cowpeas), can become discolored and suffer undesirable changes in texture (they become mushy) if they have been damaged by insects before cooking (for example, Hawkins 1980). Many canners are reluctant to accept seed lots with even modest amounts of damage (for example, 3.5 percent). Thus, it is not surprising that the lentil

industry is concerned about the consequences of chalky spot syndrome for cookability and taste of processed products (H. Blain, personal communication, 1981), which are many and varied among different ethnic groups (Leung and Salunkhe 1981).

Graders' sample lots (2.25 kg) of lentil cv. Chilean harvested in 1980 from farmers' fields close to Troy, Idaho (see fig. 1), were found to contain approximately 10 to 15 percent chalky spot seeds. The seed lots were cleaned by hand to remove inert matter and seeds of species other than lentils. Samples of each of approximately 900 g were then "reconstituted." Three samples, comprising (a) visibly undamaged seeds only; (b) 90 percent undamaged seeds, 10 percent chalky spot seeds; and (c) 80 percent undamaged and 20 percent chalky spot seeds (respective proportions by weight), provided the material for a range of cookability tests and taste panel evaluations.

Sensory, texture, and color tests were made on preparations from each of the samples described above.<sup>6</sup> Eight panelists participated in three sensory evaluations (23, 24, and 25 June 1981) of cooked samples. Four subsamples, each of 110 g, of the three seed lots were prepared for each panel. The seed lot comprised of undamaged seeds only served as a hidden control for panels 1 and 2; the lot containing 10 percent chalky spot seeds was the hidden control for panel 3.

Each subsample was rinsed with 200 ml distilled water and then placed into separate Pyrex casseroles (0.95 L capacity) along with 360 ml boiling distilled water and 2.25 g table salt. The casseroles were covered with Pyrex lids, placed on two racks in an oven preheated to 176°C, and baked for 50 min. Casseroles were rerandomized from the bottom to the top rack and from left to right positions and vice versa after 25 min of baking. Individual subsamples were drained and weighed after the baking period. All provided three portions, each of 30 ml, which were placed in 50-ml coded beakers and covered tightly with aluminum foil. The beakers were placed at random into white plastic trays containing boiling water to a depth of 4 cm to keep the samples warm. Panelists were asked to assess the difference in flavor, texture, and appearance compared with a control sample on a nine-point scale (where 1 = no difference and 9 = extreme difference).

Immediately after the sensory tests had been completed, other portions of each cooked subsample, covered in the interim, were evaluated for texture using an Allo-Kramer Shear Press (duplicate samples each of 40 g) and for color using a Hunter-Color and Color-Difference Meter (duplicate samples 1.5 cm deep in glass cylinders of 6-cm diameter).

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<sup>6</sup>Home Economics Research Centre, White Hall, Washington State University, Pullman, Wash. 99164.

## RESULTS AND DISCUSSION

### Magnitude of the Problem, Farmers' Opinions, and Pointers for Research

Between 10 and 15 percent of the Palouse lentil crop may suffer a loss in quality because of chalky spot seeds (fig. 1). Damaged seed lots are graded as U.S. No. 2, which means a penalty of about 15 percent compared with grade No. 1 seeds in their market price. Farmers have not reported a loss in economic yield, but this does not preclude the fact that yields may have been lessened. Chalky spot seeds may be the most obvious manifestation of a more general crop malaise that has yet to be identified.

The problem is worse in some years than in others and in certain areas in particular. For example, crops produced east and southeast of Moscow, Idaho (around Troy, Kendrick, and Genesee), are often affected (seeds from about 50 percent of the fields harvested in this area had significant damage in 1979), whereas those grown in the region of Garfield and Palouse, Wash., are seldom damaged. These two general areas of production are only 35 to 75 km apart (fig. 2); they do not experience markedly different climates or weather patterns, nor is the soil appreciably different in physical or chemical composition (Austin 1965).

Whether the gradual increase in annual precipitation, decreases in temperature, and tendency to soils deficient in sulphur, boron, and/or molybdenum along the eastward transect from the eastern Palouse area to the Moscow mountain foothills (Horner et al. 1957) are significant over such relatively short distances is not yet known. Agronomically, farming systems differ in the fact that rape crops (*Brassica napus* L.) are more commonly grown east rather than north of Moscow. This, we believe, may be an important difference. *Lygus* bugs are known to be especially attracted to the color yellow (Landis and Fox 1972): Cruciferous weeds along roadsides and ditches, in orchards, and on fallow and wasteland are major spring hosts of overwintering *Lygus* bug females (Fye 1980); and rape has been cited (along with carrots, radish, mustard, and collards) as a cultivated host to which *Lygus* will migrate from adjacent weed flora during the late spring and summer in southwestern Washington (Hagel 1978; and see Auld et al. 1980).

Collectively, this circumstantial evidence suggests that there may be good ecological reasons why populations of *Lygus* have developed and persisted in the area east of Moscow, and that the sequence of growth and death of annual Cruciferous weeds, flowering in rape and then flowering in lentils may provide a prolonged source of nourishment to which the pest is particularly attracted. Then again, lentil fields in the cooler, wetter areas east and southeast of Moscow are more commonly harvested as a standing crop (as opposed to swathing), and forage crops (for example, clovers and alfalfa) are grown more frequently in this area than they are in the region of Garfield and Palouse. Thus, not only are other crops to which *Lygus* bugs are attracted (from whence they can migrate to lentils) more prevalent in the area around Moscow, but also lentils are sown later and are present in fields for longer into the calendar year than they are further north.





Figure 1.--Seeds of lentil cv. 'Chilean'. Upper photograph shows seeds damaged (left) and not damaged (right) by chalky spot syndrome; lower photograph shows seeds damaged (above) and not damaged (below) by chalky spot syndrome. Lines are a 1-cm<sup>2</sup> grid.

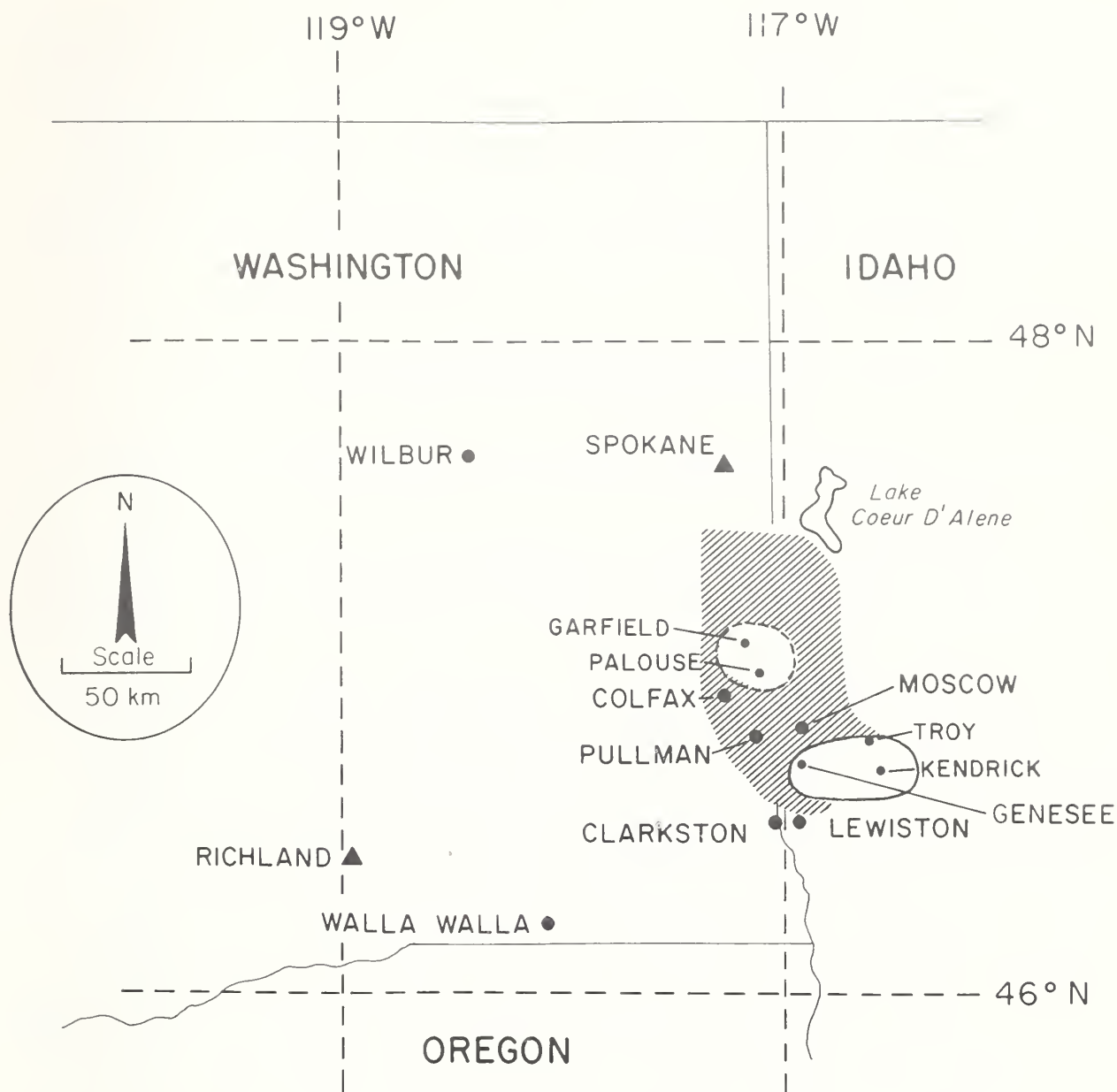


Figure 2.--Location of U.S. lentil production (hatched); where crops are known to be damaged by chalky spot syndrome (—); and areas seldom affected by the problem (-----).

In attributing significance to any geographical differences in pest incidence, we have to assume that graders of seeds in different areas adopt equally stringent standards. This assumption is not beyond question for lentil seeds from Palouse crops since subjective decisions are involved in the grading process (H. Blain, personal communication, 1981).

Farmers differ widely in their opinions as to the most probable cause of chalky spot: parochial theories blame the weather, which is either too hot or

too cold, or too wet or too dry at one particular growth stage or another. Soils too heavy or too light in texture and "nutrient disorders" have also been implicated. Some producers insist that more seeds are damaged if the lentils are harvested as a standing crop rather than swathed. Of course, each of these factors could interact with another hitherto unsuspected cause, bringing the incidence of chalky spot to a threshold value at which it becomes noticed by producers. What is clear is that lentil producers whose crops suffer chalky spot have no convincing evidence that supports one probable cause as opposed to any other. We believe that infestation by *Lygus* bugs merits serious consideration in any attempts to resolve the problem.

## Physical, Chemical, and Physiological Characteristics of Damaged and Undamaged Seed Lots

### Interseed and Intraseed Dry Matter Distribution, Germinability, and Vigor

The consequences of chalky spot syndrome were substantially the same in all four cultivars (table 2), and so it is possible to present overall average values of the attributes investigated to show the responses of these genotypes as a group (table 3).

The incidence of chalky spot was not confined to seeds of any particular size, nor did it result in the formation of seeds of similar size, but it virtually precluded the formation of large seeds and greatly increased the prevalence of relatively smaller seeds in the population (fig. 3). For example, damaged seeds heavier than 60 mg and undamaged ones lighter than 35 mg were rare, whereas healthy seeds in excess of 65 mg and damaged ones lighter than 30 mg were quite common (fig. 3). Thus, apart from any effects on seed quality, chalky spot also reduced seed dry weight because it limited the ability of seeds to realize their full growth potential. Interestingly, accession LC711981, which has a proven ability to outyield current commercial cultivars in the field (Muehlbauer 1981), seems better able to produce larger seeds when damaged by chalky spot than the other cultivars tested here (table 2). We made no attempt, however, to quantify the degree of chalky spot damage on either individual seeds or cultivars, or to assess the overall prevalence of damaged seeds in the bulk lots from which these samples were collected. Hence, the tolerance or resistance of LC711981 to chalky spot syndrome remains to be verified once the cause of malformation is proven.

Single punctures of *Phaseolus* seeds by *Lygus* bugs have reduced average seed dry weight by 18 percent compared with undamaged seeds, and three or four punctures per seed are even more deleterious (average reduction in weight of 36 percent; Scott 1970). The relative magnitudes of these deprivations are remarkably similar to those caused by chalky spot on lentils (table 2). Scott (1970) reasoned that seed weights were reduced because normal embryogenesis had been prevented ("stunted"), and that lysis of endosperm by salivary secretions was followed by siphoning of partially digested materials by the feeding insect pests.

The losses in seed dry matter reported here (tables 2 and 3) are likely to be underestimates of the true magnitude of the problem. For example, seeds

Table 2.--Attributes of lentil seeds damaged (+) or not (-) by chalky spot syndrome. All seeds collected at random from bulk samples of lots harvested in 1980 from a single location

Attribute measured or derived	Seed stock (USDA germplasm accession)									
	LC711981		VW000457		Redchief		VW000563			
	+	-	+	-	+	-	+	-	+	-
Water content (percent) <sup>1</sup>	7.69±0.04	6.48±0.05	7.62±0.09	6.69±0.04	7.81±0.07	6.53±0.07	7.54±0.10	6.99±0.07		
Dry weight (g) of 1000 seeds <sup>2</sup>	48.1	55.1	37.7	52.3	31.5	49.7	31.4	45.0		
Loss in weight (per- cent) due to chalky spot		12.8		27.9		36.7		30.3		
Median seed weight (mg range) <sup>2</sup>	51-55	61-65	41-45	56-60	31-35	51-55	41-45	51-55		
Mean seed dry weight (mg) <sup>3</sup>	48.8±.16	50.0±.14	47.4±.19	51.5±.39	48.5±.60	49.4±.14	47.8±.34	48.8±.54		
Mean cotyledon dry weight (mg)	40.5±.40	44.0±.24	40.0±.16	45.4±.29	39.7±.53	43.5±.16	39.3±.63	43.4±.54		
Mean testa dry weight (mg)	8.3±.27	6.1±.20	7.4±.19	6.0±.12	8.8±.20	5.9±.04	8.5±.33	5.4±.07		
Proportion (percent) of whole seed dry weight as testa <sup>4</sup>	17.0±.59	12.1±.38	15.6±.37	11.8±.16	18.2±1.26	12.0±.10	17.7±.70	11.2±.19		

See footnotes at end of table.

Table 2.--Attributes of lentils seeds damaged (+) or not (-) by chalky spot syndrome. All seeds collected at random from bulk samples of lots harvested in 1980 from a single location--Continued

Attribute measured or derived	Seed stock (USDA germplasm accession)									
	LC711981					VW000457				
	+	-	+	-	+	+	-	+	-	+
Percent germination (5 days)	76.4± 4.5	83.3± 7.0	81.2± 2.2	94.8± 0.7	73.2± 7.4	90.3± 1.8	88.3± 3.4	92.5± 1.9		
Percent germination (10 days) <sup>5</sup>	77.4± 4.0	95.6± 2.0	81.8± 2.8	95.6± 1.3	77.3± 9.3	93.3± 2.9	90.0± 2.9	94.2± 1.1		
Seeds without embryos (percent) <sup>6</sup>	10±0	0	0	0	9.3± 0.3	0	9.7± 0.3	0		

<sup>1</sup>Expressed as percent fresh weight; 5 replicates of 20 seeds; means ± standard errors.

<sup>2</sup>And see figure 2.

<sup>3</sup>All seeds from 51 to 55 mg fresh weight class.

<sup>4</sup>All raw and derived data based on 5 replicates of 20 seeds; means ± standard errors.

<sup>5</sup>Means ± standard errors based on 5 replicates of 30 seeds.

<sup>6</sup>Means ± standard errors based on 3 replicates of 10 seeds.



Table 3.--Overall average valued ( $\pm$ SE) of selected attributes of lentil seeds damaged or not by chalky spot syndrome (mean values of all cultivars in table 2)

Attribute	Seed with chalky spot	Seeds without chalky spot
Water content (percent) <sup>1</sup>	7.67 $\pm$ 0.07	6.76 $\pm$ 0.06
1,000 seed dry weight (g) <sup>2</sup>	37.2 $\pm$ 3.9	50.5 $\pm$ 2.1
Loss in weight (percent) due to chalky spot	26.9	5.1
Median seed weight (mg range) <sup>3</sup>	41-45	56-60
Mean seed dry weight (mg) <sup>4</sup>	48.1 $\pm$ .32	49.9 $\pm$ .58
Mean cotyledon dry weight (mg)	39.9 $\pm$ .26	44.1 $\pm$ .46
Mean testa dry weight (mg)	8.3 $\pm$ .30	5.9 $\pm$ .16
Proportion (percent) of whole seed dry weight in testa	17.1 $\pm$ .56	11.8 $\pm$ .20
Percent germination (5 days)	79.8 $\pm$ 4.4	90.2 $\pm$ 2.9
Percent germination (10 days)	81.6 $\pm$ 4.8	94.7 $\pm$ 2.1
Seeds without embryos (percent)	5.0 $\pm$ 2.2	0

<sup>1</sup>Expressed as percent fresh weight.

<sup>2</sup>See figure 2.

<sup>3</sup>Approximated to the nearest weight class as used in figure 2.

<sup>4</sup>All seeds from 51 to 55 mg (fresh) weight class.

lighter than, say, 20 mg (reflecting, perhaps, acute development of chalky spot and/or incidence of the problem at a particularly critical stage of seed ontogeny; see Dure 1975) are likely to be easily lost from the combine at harvest.

Although they were harvested to within one day of each other, and then placed promptly into storage in identical paper sacks at the same temperature (10° to 13°C in the dark), the damaged seeds 3 months later had larger water contents (7.67 $\pm$ 0.7 percent) than undamaged ones (6.76 $\pm$ 0.06 percent). Physical damage to the seedcoat could conceivably allow water to penetrate but, of course, to escape as well. Whether this significant difference in water content reflects markedly different physical endostructures (for example, in the integrity of cellular membranes) and, therefore, physiological activities (for example, respiration rate), and also renders damaged seeds more prone to deterioration in storage (Roberts 1972), remains to be tested. In general, at least with seed moisture contents down to about 5 percent, each 1-percent reduction in seed moisture doubles the storage life of the seed and vice versa (Harrington 1973).

Certainly, damaged seeds were better able to imbibe water than healthy ones. The few undamaged seeds that had not germinated after 10 days in the germination test (tables 2 and 3) had failed to imbibe water (they were described as "hard-seeded") whereas all the seeds with chalky spot were fully im-

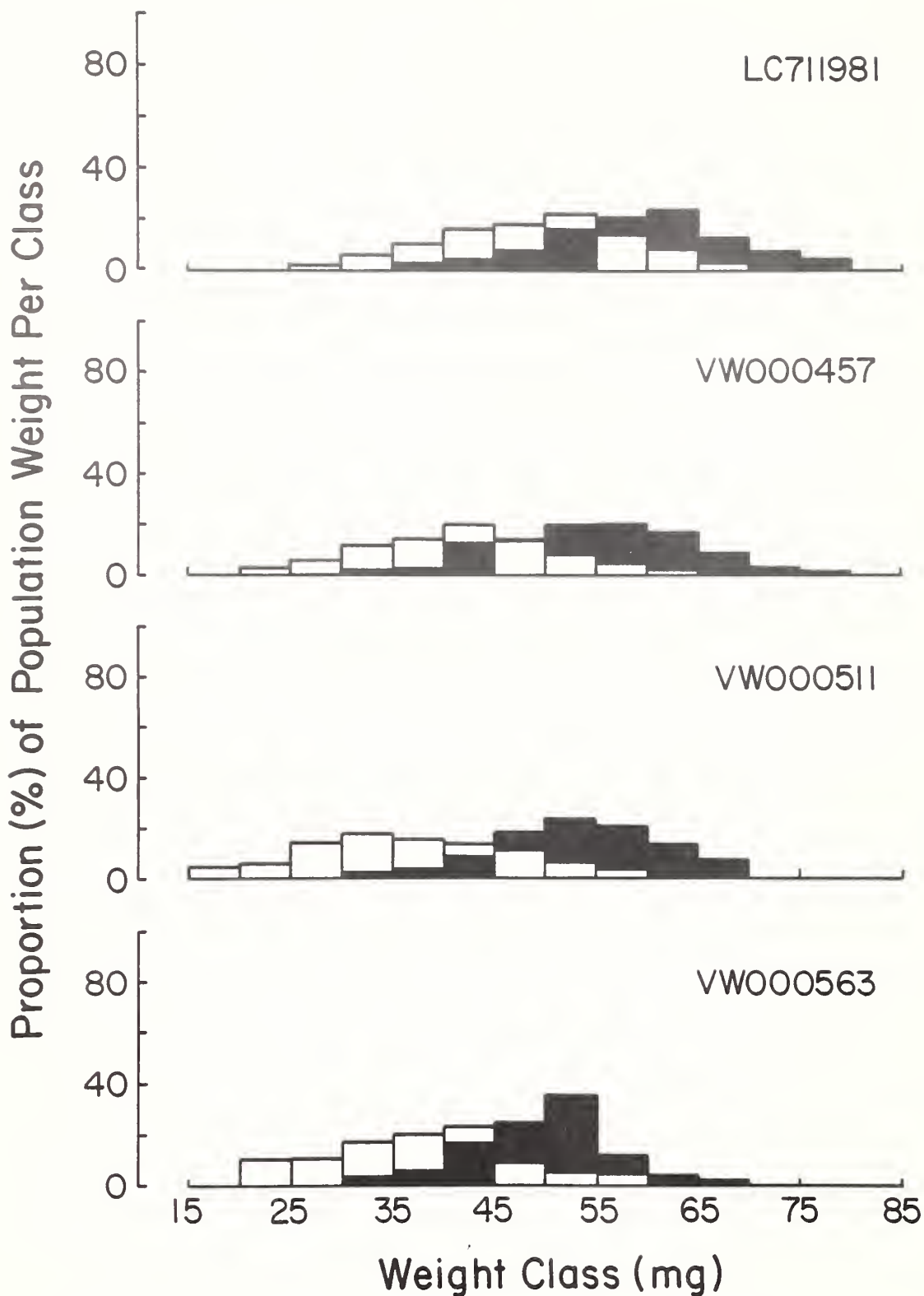


Figure 3.--Frequency distributions for lentil seeds of various weights (mg range) within populations damaged (open) or not (shaded) by chalky spot syndrome.



bibed and swollen. The failure of a larger proportion of damaged seeds to germinate is attributed to two principal causes: heavy infestations of fungal pathogens and absence of embryos.

The fungal contaminants of nongerminated, chalky spot seeds were identified as *Fusarium* spp., *Cladosporium* spp., and *Alternaria* spp. (collectively the most prevalent species) together with *Mucor* spp., *Penicillium* spp., and *Stemphylium* spp. These species of fungi are components of a microflora that might reasonably be expected to colonize seeds either developing on plants in the field, or after the seeds have matured and the plants are either still standing or have been cut and swathed, awaiting threshing (Christensen 1972). We did not, however, record fungal growth on any healthy seeds during the time scale of this experiment. Chalky spot damage may have allowed exudation or leakage of energy-rich cotyledon reserves during imbibition, which then stimulated spore germination and hyphal growth (rather than inherent differences in the fungal flora of damaged and healthy seeds). Indeed, as table 4 shows, damaged seeds were an average of three times more leaky than undamaged ones. Again, accession LC711981 seems outstandingly better than the other three cultivars in that although damaged seeds leaked more of their storage reserves than healthy ones, the conductivity value of steep water was 38-percent smaller than the average value for chalky spot seeds of the other cultivars. As we stressed earlier, however, the relative resistance of LC711981 to chalky spot has yet to be proven. (Its undamaged seeds were slightly inferior in leakability to the other three cultivars.) In selecting for larger yields (Muehlbauer 1981), the breeder may have also unconsciously selected for an ability to resist, tolerate, escape, compensate, or recover from chalky spot syndrome.

The increase in acidity of steep water (an average decline of 1.44 and 0.84 units of pH for damaged and healthy seeds, respectively) probably reflects leakage of amino, carboxylic, and fatty acids--all of which are known to be readily leached from seeds of other legume species within a time scale similar to the one used here (J. D. Maguire, personal communication, 1981).

It may be pertinent to note here that alfalfa seeds (*Medicago sativa*) challenged by *Lygus* bugs, and often with physical deformations remarkably similar to chalky spot on lentils, may fail to germinate when planted (for example, Johansen and Retan 1975); and that *Lygus* bug damage to lima beans (*Phaseolus lunatus*) was once attributed solely to infestation by the yeast *Nematospora phaseoli* (Wingard 1922). It was necessary, however, to puncture the lima bean pods with an infected needle to transmit the fungus (atomizer sprays and surface smears were ineffective), and seeds were more susceptible to damage before they had attained one-half their normal mature size. These observations, plus the fact that pod walls were seldom damaged visibly (albeit they may have enclosed badly diseased seeds), and that farmers' crops could suffer anywhere between "a trace" and 60-percent infection, are in parallel with other findings for *Lygus*-infected crops in general (Johansen and Retan 1975; Wingard 1922; Christensen 1972; Shull and Wakeland 1931) and, in particular, with the incidence of chalky spot on lentils described here.

Lentil embryos typically represent slightly less than 2 percent of seed dry weight (Singh et al. 1968) and are easily visible with the naked eye when cotyledons are separated. Each of the 120 undamaged seeds examined here contained an apparently healthy embryo, whereas an average of 5.0 (2.2 percent) of chalky

Table 4.--Electrical conductivity and acidity of steep water from lentil seeds damaged (+) or not (-) by chalky spot syndrome (mean values of 4 replicates  $\pm$ SE)

Attribute	Seed stock (USDA accession)									
	Control		LC711981		VW000457		Redchief		VW000563	
	+	-	+	-	+	-	+	-	+	-
Weight of seeds used (g)	0	2.93 $\pm$ 1.10	2.98 $\pm$ 0.04	1.81 $\pm$ 0.06	3.25 $\pm$ 0.05	1.77 $\pm$ 0.06	3.05 $\pm$ 0.06	1.79 $\pm$ 0.06	2.75 $\pm$ 0.07	
Initial pH of steep water	6.28 $\pm$ .14	6.43 $\pm$ .08	6.58 $\pm$ .25	6.39 $\pm$ .21	6.59 $\pm$ .19	6.01 $\pm$ .11	5.91 $\pm$ .08	5.90 $\pm$ .08	6.40 $\pm$ .10	
Initial conductivity <sup>1</sup> of steep water	1.37 $\pm$ .22	1.24 $\pm$ .13	2.31 $\pm$ .75	1.62 $\pm$ .51	2.08 $\pm$ .40	.97 $\pm$ .03	.93 $\pm$ .01	.97 $\pm$ .04	1.54 $\pm$ .09	
Final pH of steep water after 24 h	6.38 $\pm$ .09	4.85 $\pm$ .04	5.40 $\pm$ .05	4.62 $\pm$ .03	5.49 $\pm$ .16	4.74 $\pm$ .04	5.71 $\pm$ .10	4.75 $\pm$ .12	5.53 $\pm$ .06	
Final conductivity <sup>1</sup> of steep water after 24 h	2.28 $\pm$ .27	123 $\pm$ 6	68 $\pm$ 5	129 $\pm$ 7	62 $\pm$ 5	120 $\pm$ 12	51 $\pm$ 2	112 $\pm$ 4	50 $\pm$ 1	
Corrected final pH <sup>2</sup>	-	4.75	5.30	4.52	5.39	4.64	5.61	4.65	5.43	
Corrected final conductivity <sup>2</sup>	-	122	67	128	61	119	50	111	49	
Electrical conductivity per unit weight of seeds ( $\mu$ mhos cm <sup>-2</sup> g <sup>-1</sup> )	-	41.6	22.5	70.7	18.8	67.2	16.4	62.0	17.8	

<sup>1</sup>Conductivity bridge Model RC 16B2, Industrial Instruments, Inc.

<sup>2</sup>Adjusted values to compensate for drift in control samples.

spot seeds did not (tables 2 and 3). The formation of embryoless seeds is symptomatic of *Lygus* bug damage to carrots (*Daucus carota*), dill (*Anethum graveolens*), and fennel (*Foeniculum dulce*); as many as 100 percent of seeds can be affected in forced-feeding situations (for example, Flemion et al. 1949; Flemion and Olsen 1950). Hence, although the larger seeds of lentils seem less likely to suffer a loss of embryos than these smaller-seeded Umbelliferous species, the fact that a small proportion of seeds had been killed by chalky spot because their embryos had been destroyed is further evidence implicating *Lygus* bugs, or an insect with similar mouth parts and feeding niches, as the causal agent of chalky spot syndrome.

Subsequent research needs to evaluate the effects of mechanical damage on seed viability in lentils (for example, see Moore 1972) and to ensure that physical defects in seeds and seedlings are due to mechanical and not to entomological causes. As table 5 shows, seedling emergence from chalky spotted seeds was delayed and then dramatically poorer than from healthy seeds (average values of 20- and 86-percent emergence, respectively). Furthermore, a large proportion of seedlings that did eventually emerge was abnormal morphologically (fig. 4). Since all seeds were surface sterilized with sodium hypochlorite (1 percent a.i.) before sowing, the poor performance of those damaged by chalky spot may have been due, at least in part, to penetration of the sterilant. Nevertheless, since farmers often dress their lentil seeds with fungicides (Summerfield et al. 1982), we might expect the likelihood of seedling emergence from chalky spotted seeds in the field to be similarly poor.

Although relatively poor and/or delayed germination of *Lygus*-damaged seeds has been reported for several species (for example, Carlson 1956), the consequences need not be altogether unfavorable. Plants raised from damaged carrot and bean seeds produced heavier roots and larger seed yields, respectively, than those raised from healthy seeds (Scott 1970). Several hypotheses were advanced to explain these surprising differences, but Scott (1970) favored a theory that involved injection of an auxin by the feeding bugs, which are known to secrete materials into green bean pod tissues as they feed (Flemion et al. 1952). It is difficult to envisage what mechanism(s) might have evoked such persistent effects because those described by Scott and his data are not unequivocal.

In general, developing seeds are particularly rich sources of auxin, mature seeds less so, and it is not clear what role, if any, auxin plays in germination (Bewley and Black 1978). As tables 2 and 3 show, damaged lentil seeds have significantly heavier testa (which also represent a significantly larger proportion of whole seed dry matter) than healthy ones, and any interrelationships between endogenous hormone concentrations and factors that shift the normal path of embryogenesis in favor of testa formation can only be speculative. If embryogenesis in lentils proceeds as in peas (*Pisum sativum*) and beans (*Phaseolus vulgaris*), which seems likely (see Dure 1975), then localized damage relatively soon after anthesis (when cell division is most rapid) could conceivably promote dry matter investment into testa at the expense of cotyledons. The course of embryogenesis in lentil seeds, healthy or otherwise, has yet to be described.

There is an adage often repeated among farmers: "The crop can be no better than the seed sown." Seed quality should probably encompass at least four major attributes; (a) genetic purity, especially important for crop uniformity at maturity; (b) physical purity, freedom from inert matter and other unwanted

Table 5.--Seedling establishment (percent emergence) and prevalence of seedlings with abnormal morphology (percent of those emerged) for lentil cultivars grown from visibly healthy (-) or chalky spotted (+) seeds<sup>1</sup>

Attribute	Seed stock (USDA accession)							
	LC711981		VW000457		Redchief		VW000563	
	+	-	+	-	+	-	+	-
Percent emergence (5 days)	2±1	48±3	12±4	68±5	4±3	64±5	6±2	71±4
Percent emergence (10 days)	20±4	76±5	25±5	92±3	19±5	87±3	17±4	88±3
Percent abnormal seedlings	55±3	0	48±3	0	79±4	0	35±3	0

<sup>1</sup>Mean values of 10 replicates (pots) each of 10 seeds sown ± SE.



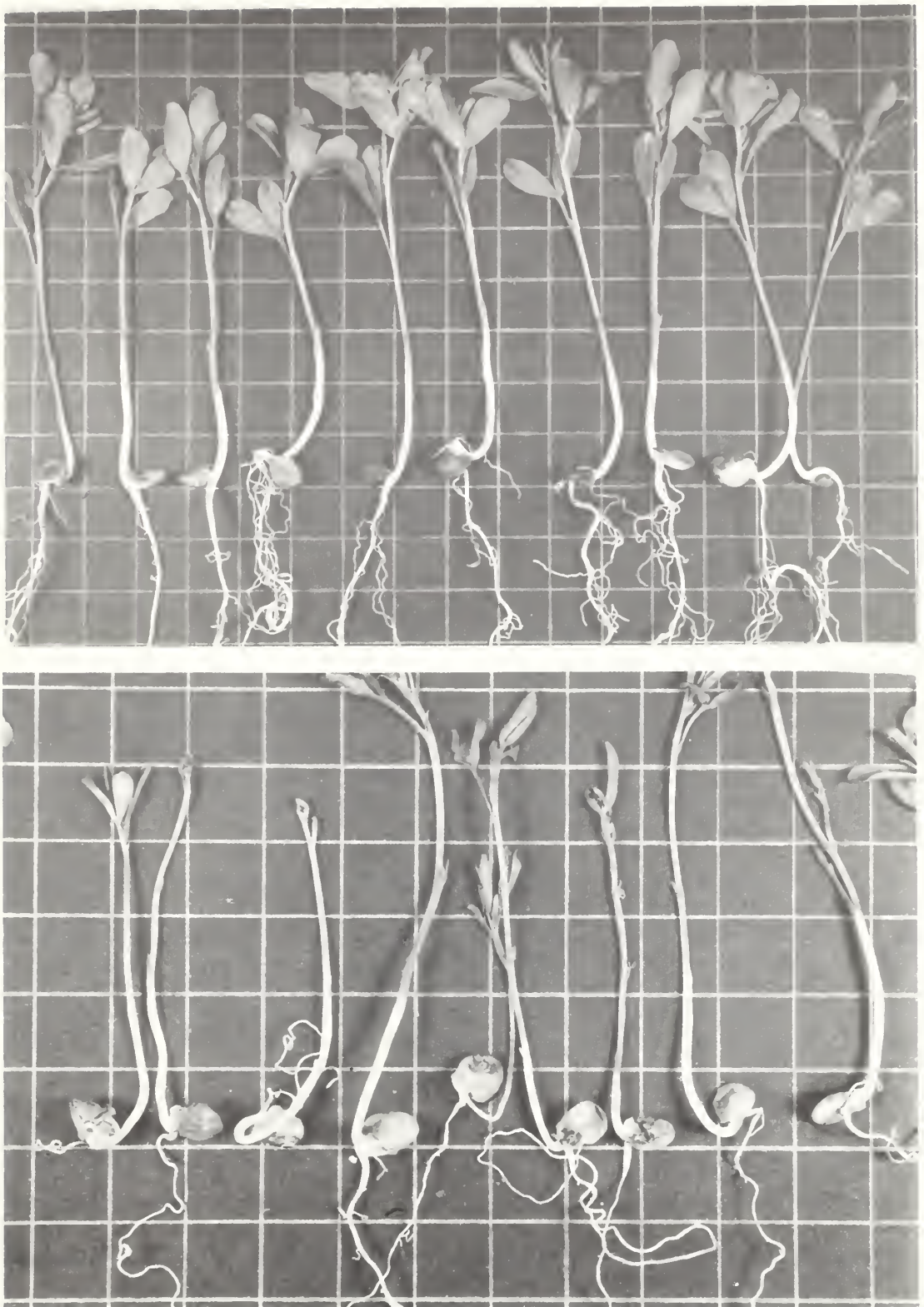


Figure 4.--Lentil seedlings harvested 10 days after sowing visibly healthy seeds (above) or ones damaged by chalky spot (below) in pots in a glasshouse (lines are a 1-cm<sup>2</sup> grid).

seeds; (c) large germination potential, at least 85 percent germinability; and (d) abundant vigor, so that seedlings emerge rapidly and uniformly over a wide range of seedbed conditions (Roberts and Ellis 1980). The first two attributes are easily understood (but the principles of control are not always put into practice), the third refers to the proportion of seeds capable of producing normal seedlings under ideal conditions, but the fourth attribute, vigor, is much more problematical. It should perhaps be considered as a concept rather than an attribute amenable to quantitative description (Roberts and Ellis 1980). Chalky spot syndrome of lentils potentially affects only the latter two attributes of quality--germinability and vigor. However, since lentils are used almost exclusively for food rather than, for example, oil extraction and for animal feed, then, as in *Phaseolus* beans, other attributes of quality must be considered.

Although some producers argue that "chalky insect stings" on cowpea seeds are of little concern to canners, packagers, or consumers, some canners will reject all damaged lots outright and others will not accept a seed lot if the damage exceeds 3.5 percent (Hawkins 1980). Chalky spot stings on cowpea appear as dark brown spots after cooking, and damaged seeds may also become mushy in texture. The effects of chalky spot syndrome on cookability of lentil seeds are described on page 33.

## Chemical Composition

The chemical compositions of damaged and undamaged seeds are presented in table 6. At first sight, it may appear that seeds with chalky spot syndrome are relatively enriched with each of these elements compared with visibly normal seeds. However, we believe that these are systematic differences consequent upon the nature of the samples analyzed.

Although only seeds of uniform size (41 to 45 mg) constituted the samples analyzed, they did not comprise equal proportions of cotyledons and testa: unit weight of chalky spot seeds comprised a smaller proportion of cotyledons and a correspondingly larger proportion of testa than in undamaged seeds (table 3). Since cotyledons and embryos together contain by far the largest proportions of the elements assimilated into lentil seeds (for example, 97.8, 96.0, 84.3, and 64.5 percent of P, N, Fe, and Ca, respectively; Singh et al. 1968), then an identical absolute amount of a given element in damaged and undamaged seeds would appear more concentrated (percent) in samples of the former than those of the latter. Thus, unless seeds are decorticated beforehand, data on mineral nutrient composition should not be compared and contrasted directly, either within or between treatments or genotypes, without additional knowledge of intraseed dry matter distribution.

Applying an appropriate correction factor (multiply by 0.940) to the data presented for chalky spot seeds in table 6 to allow for differences in the proportion of samples in cotyledons, yields a more realistic picture of the consequences of chalky spot on nutrient composition (table 7). From these revised estimates, only differences in Ca concentration seem to be larger than expected from random intersample variability (table 7). Again, we favor an explanation based on differences in dry matter and nutrient partitioning within seeds rather

Table 6.--Chemical composition (percent; with parts per million in parentheses) of oven-dried lentil seeds damaged or not by chalky spot syndrome (mean values  $\pm$ SE of 4 replicates of bulked samples of 4 varieties)

Element	Concentration of element in whole seeds <sup>1</sup>			
	With chalky spot		Without chalky spot	
N	5.10	$\pm 0.02$ (5.1x10 <sup>4</sup> )	4.75	$\pm 0.02$ (4.8x10 <sup>4</sup> )
P	.608	$\pm .19$ (6.1x10 <sup>3</sup> )	.542	$\pm .008$ (5.4x10 <sup>3</sup> )
K	1.189	$\pm .024$ (1.2x10 <sup>4</sup> )	1.070	$\pm .022$ (1.1x10 <sup>4</sup> )
Ca	.079	$\pm .001$ (790)	.064	$\pm .0007$ (640)
Mg	.133	$\pm .003$ (1.3x10 <sup>3</sup> )	.124	$\pm .002$ (1.2x10 <sup>3</sup> )
S	.228	$\pm .005$ (2.3x10 <sup>3</sup> )	.208	$\pm .004$ (2.1x10 <sup>3</sup> )
Fe	.0098	$\pm .0003$ (98)	.0090	$\pm$ (90)

<sup>1</sup>Cotyledons, embryos, and testa combined.

Table 7.--Chemical composition (percent; with parts per million in parentheses) of oven-dried lentil seeds damaged or not by chalky spot syndrome (estimates for samples comprising the same proportions of cotyledons and testa; mean values  $\pm$ SE based on 4 replicates)

Element	Concentration of element in whole seeds <sup>1</sup>			
	With chalky spot		Without chalky spot	
N	4.79	$\pm 0.02$ (4.8x10 <sup>4</sup> )	4.75	$\pm 0.02$ (4.8x10 <sup>4</sup> )
P	.571	$\pm .013$ (5.7x10 <sup>3</sup> )	.542	$\pm .008$ (5.4x10 <sup>3</sup> )
K	1.116	$\pm .028$ (1.1x10 <sup>4</sup> )	1.070	$\pm .022$ (1.1x10 <sup>4</sup> )
Ca	.073	$\pm .0009$ (730)	.064	$\pm .0007$ (640)
Mg	.125	$\pm .003$ (1.3x10 <sup>3</sup> )	.124	$\pm .0002$ (1.2x10 <sup>3</sup> )
S	.214	$\pm .004$ (2.1x10 <sup>3</sup> )	.208	$\pm .004$ (2.1x10 <sup>3</sup> )
Fe	.0092	$\pm .0002$ (92)	.0090	$\pm 0$ (90)

<sup>1</sup>Cotyledons, embryos, and testa combined.

than one that implicates a direct effect of chalky spot syndrome on mineral nutrition. For a cultivar with an identical concentration of N in whole seeds to the undamaged ones analyzed here (4.75 percent), but which invested only 8.05 percent of seed dry matter into testa (compare with 11.8 percent here), the



cotyledons contained only 64.5 percent of the Ca in whole seeds (Singh et al. 1968). Assuming similar distributions for this element in the present study, between 52 and 75 percent of seed calcium was likely to have been located in testa. By far, the largest proportions (80 to 90 percent or more) of all of the other elements listed in tables 6 and 7 will have been assimilated into cotyledons. Thus, applying a correction factor to account for intraseed differences in dry matter distribution is unlikely to redress the atypical bias of Ca storage in testa rather than in cotyledons.

Mineral deficiencies in seeds are likely to be rare (Austin 1972), and when they do occur it seems that the adaxial surfaces of cotyledons are more likely to show deficiency symptoms than the abaxial ones (for example, marsh spot in peas, due to Mn deficiency; hollow heart and dark plumule in groundnuts, due to B and Ca deficiency, respectively; Pollock and Roos 1972). Thus, it is not only difficult conceptually to envisage that chalky spot syndrome of lentils is the result of a nutrient disorder (either one of deficiency or excess), but the data now at hand (table 7) also support this contention.

### Microscopy

Scanning and transmission electron micrographs of damaged compared with healthy cotyledons revealed the dramatic consequences of chalky spot syndrome for cellular integrity. The epidermal and mesophyll characteristics of the abaxial surface of healthy cotyledons (fig. 5; and Patel et al. 1979) were destroyed, cotyledonary cell walls were distorted, cellular contents were disrupted, starch grains were absent, and tissue integrity was lost (fig. 6). Healthy cotyledonary cells of fully expanded, but not yet desiccated, seeds were uniformly granulate and with a proliferation of rough endoplasmic reticulum (rer) adjacent to cell walls (fig. 7). On the contrary, damaged cells had little rer and likely had very limited capacity for synthesis of storage proteins (Boulter 1977). The large intracellular air spaces of the damaged cells contained osmiophilic granules or droplets that were never seen in undamaged cells (fig. 8). The granules may have been "congealed" cellular contents, "coagulated" membrane, or even extra-cellular in origin.

This type of damage is in keeping with that expected from *Lygus* bug feeding (Flemion et al. 1952; 1954), during which the needlelike, extremely flexible stylets (fig. 9) move rapidly in all directions both inter- and intracellularly.

### Caging Lygus Bugs Onto Pot-Grown Plants

The caged plants had senesced and died after 51 to 65 days from the appearance of first flowers. Cultivar Precoz had the shortest crop duration and cv. Chilean the longest. Exposing plants to *Lygus* bugs did not affect crop longevity.

Adult bugs were frequently observed to feed on the caged plants and, in this forced feeding situation, seemed especially attracted to immature reproduc-

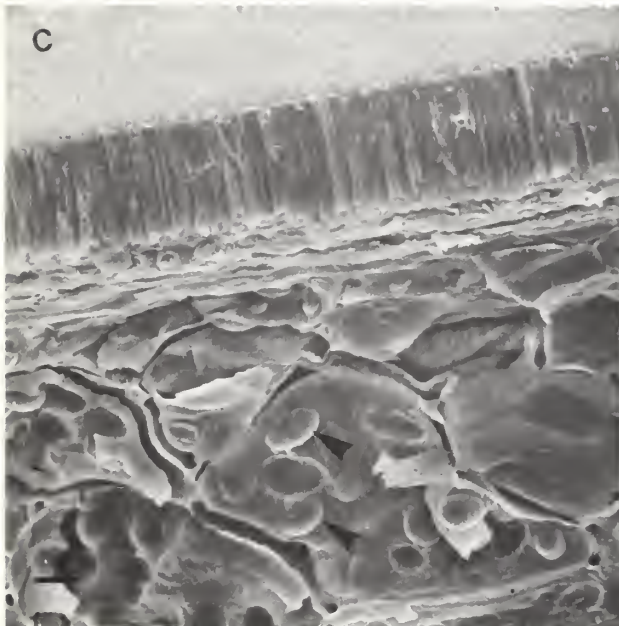
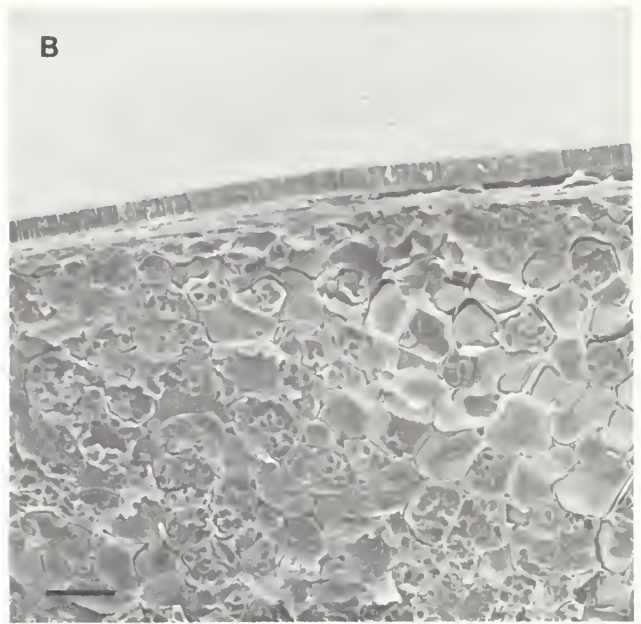
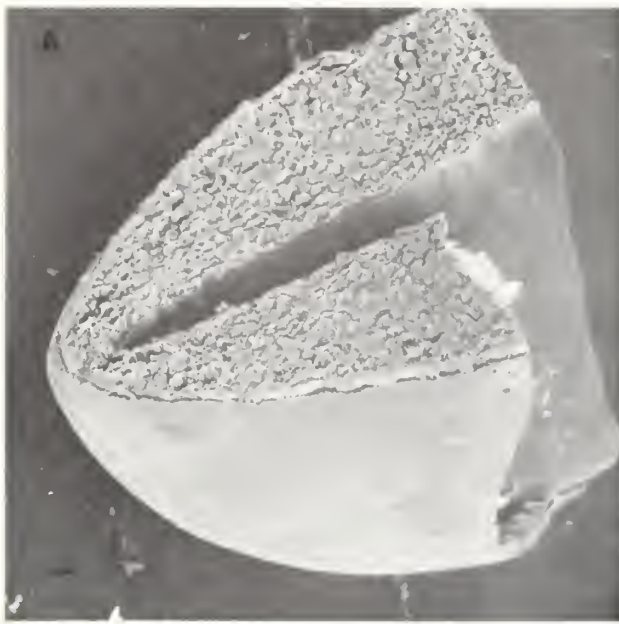


Figure 5.--Typical scanning electron micrographs of cryofractured tissues of undamaged, visibly healthy seeds: (A) Cut face of mature seed (x30); bar = 100  $\mu$ m. (B) Fractured face of mature seed (x90); bar = 100  $\mu$ m. (C) As B but x500 and bar 10  $\mu$ m; arrow-heads indicate objects between 2 and 7  $\mu$ m long, probably starch granules (Bhatti and Slinkard 1979; Morad et al. 1980).

tive structures (fig. 10). We also interpret the presence of localized droplets and stains on green pod walls to be evidence of feeding activity (fig. 11).

That the adult bugs had reproduced during the caging period is beyond doubt; all stages of the *Lygus* bug life cycle were identified at one time or another (G. R. Pesho, personal communication, 1981). Thus, we cannot be certain of the proportions of any deprecations on lentil host plants that are caused by adults or immature bugs (see citations in Flemion et al. 1949) or, indeed, whether male or female adults are likely to be relatively more destructive. Furthermore, since the original population comprised both *L. hesperus* and *L. elisus* (about 90 and 10 percent, respectively) there may be a species component involved too. Our purpose was to see if the presence of *Lygus* bugs on reproduc-



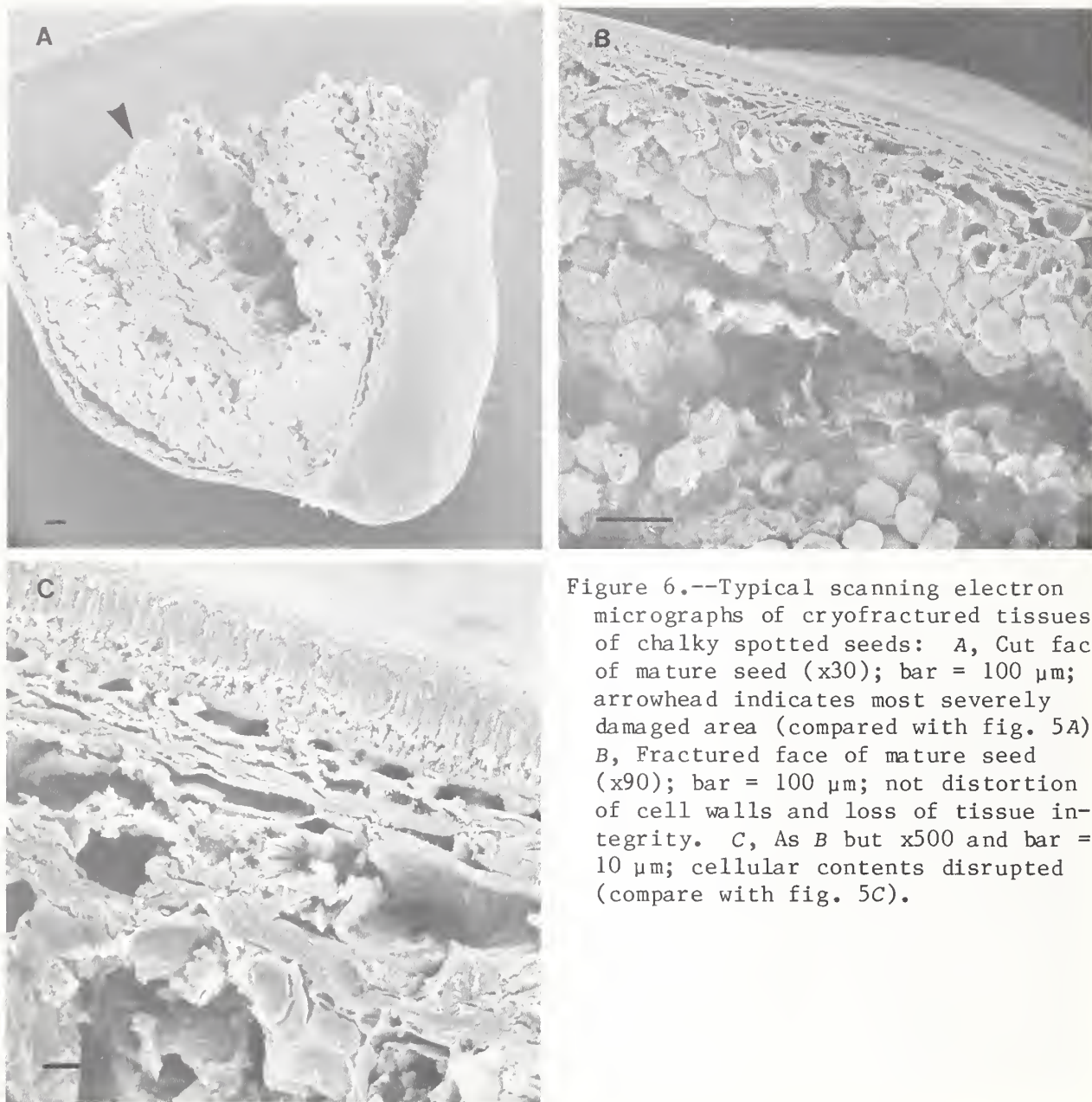


Figure 6.--Typical scanning electron micrographs of cryofractured tissues of chalky spotted seeds: A, Cut face of mature seed (x30); bar = 100  $\mu$ m; arrowhead indicates most severely damaged area (compared with fig. 5A). B, Fractured face of mature seed (x90); bar = 100  $\mu$ m; not distortion of cell walls and loss of tissue integrity. C, As B but x500 and bar = 10  $\mu$ m; cellular contents disrupted (compare with fig. 5C).

tive lentil plants was related to the formation of seeds with chalky spot syndrome. Problems such as stage of crop development when the largest proportion of plants in a stand are most vulnerable to attack, timing during embryogenesis of individual seeds when deprecations are likely to be most serious, and the species, sex, and age of *Lygus* bugs to which lentil plants are most susceptible must be researched and quantified both in the glasshouse and, especially, in the field, once cause and effect have been established beyond reasonable doubt.

Data on economic yield and selected components of yield of control plants and those subject to *Lygus* infestation are summarized in table 8 (p. 31). None of the seeds harvested from plants in the insect-free cage were damaged, whereas an average of 26 percent of those from the plants caged with *Lygus* had "classical" chalky spot syndrome. Cultivar Chilean, which produced the most nodes and

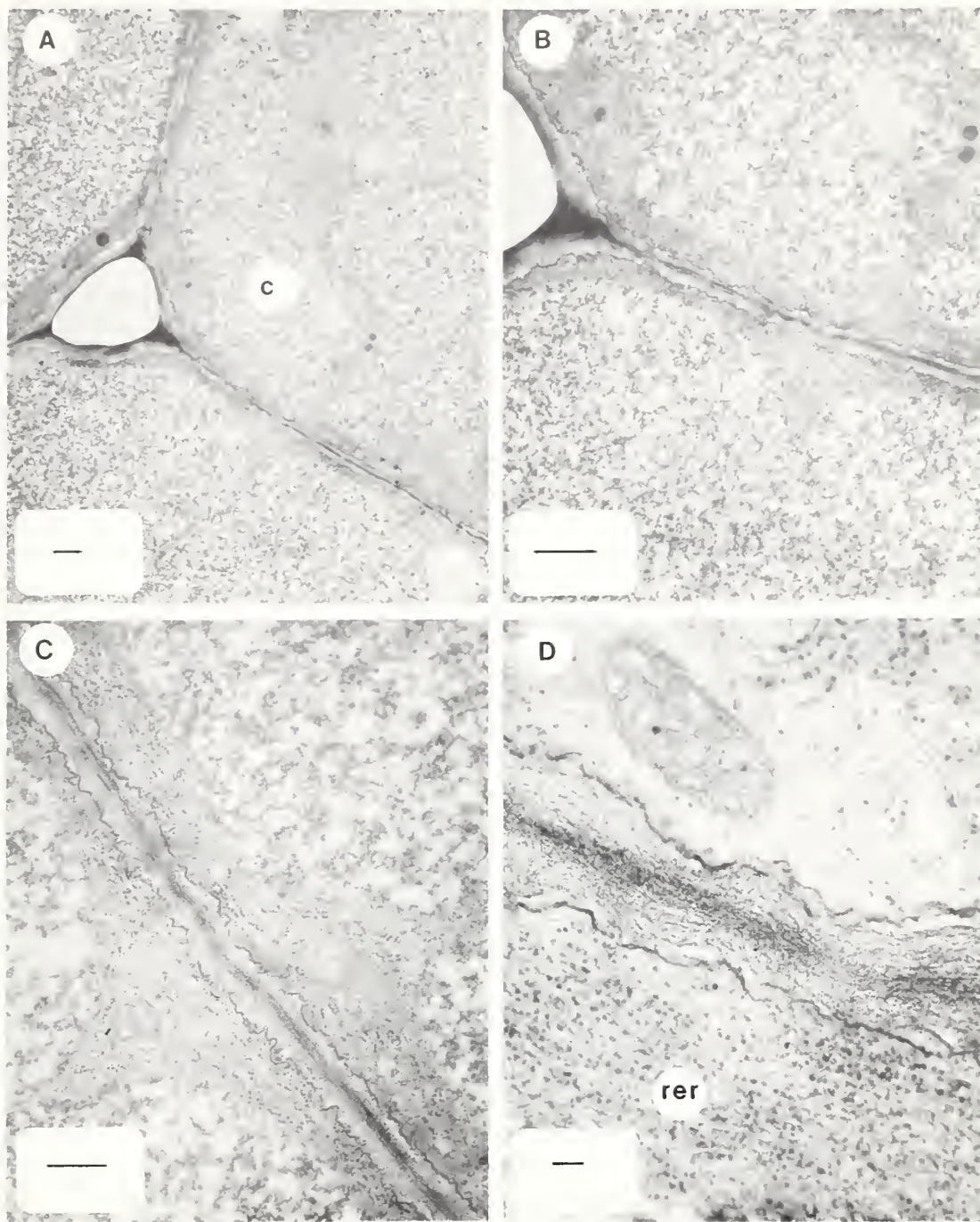


Figure 7.--Typical transmission electron micrographs of tissues of undamaged, visibly healthy seeds: A, Cells of immature cotyledons (from fully expanded, but not desiccated seeds) showing uniform distribution of seemingly granular cellular contents (c); x5,000; bar = 1  $\mu$ m. B, As A but x10,000 and bar = 1  $\mu$ m. C, As A but x20,000 and bar = 0.5  $\mu$ m. D, As A but x50,000 and bar = 0.1  $\mu$ m; note the proliferation of rough endoplasmic reticulum (rer) adjacent to cell wall.



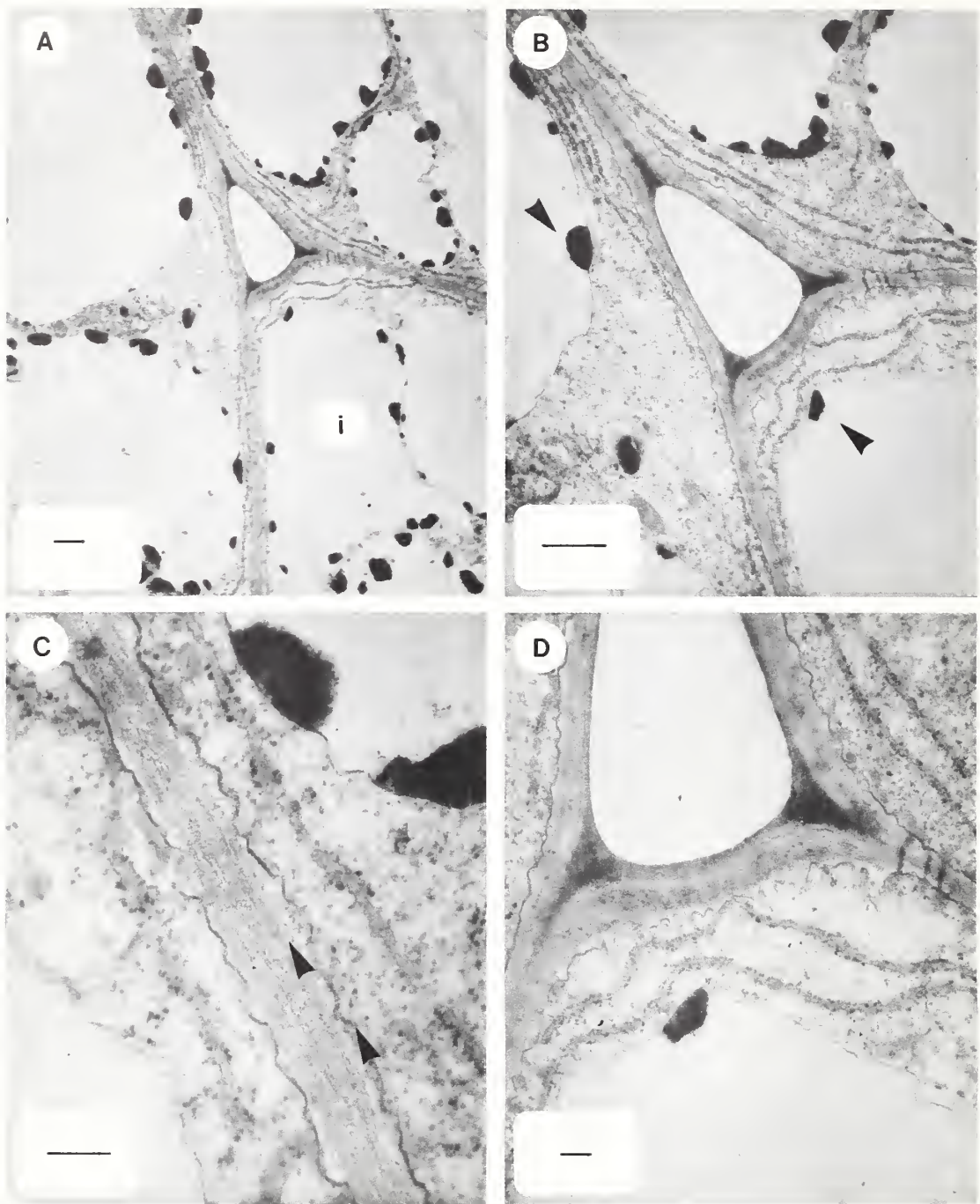


Figure 8.--Typical transmission electron micrographs of tissues of chalky spotted seeds: A, Cells of immature cotyledons (from fully expanded, but not desiccated seeds), showing large intracellular air spaces (i); x5,000; bar = 1  $\mu$ m (compare with fig. 7A). B, As A but x10,000 and bar = 1  $\mu$ m. Arrowheads indicate osmiophilic granules or droplets not present in undamaged tissues (compare with fig. 7B). C, As A but x20,000 and bar = 0.05  $\mu$ m. Arrowheads indicate stands of rer, not the masses of stacked rer cisternae seen in undamaged cells (fig. 7C, 7D). D, As A but x50,000 and bar = 0.1  $\mu$ m; note that the middle lamella, so prominent in healthy tissue (fig. 7D), is only poorly visible here.



Figure 9.--Scanning electron micrograph of adult *Lygus* bug (x40; bar = 100  $\mu$ m), showing the prominent multiple stylet. Arrowheads delimit a 1.5-mm length of stylet, which ranges between 40 and 100  $\mu$ m in width.

flowers (not recorded quantitatively here), was seemingly twice as susceptible to damage than the other cultivars (incidence of chalky spot 40.2 percent compared with an average of 18.9 percent, respectively). As expected from experience with other grain legumes (Summerfield 1980a; Summerfield and Wien 1980), not all fruits on control plants contained fully formed seeds: an average of 13.3 percent were empty and/or shrivelled (and see Sorensen 1936 for alfalfa). The presence of *Lygus* bugs, however, increased the prevalence of malformed, shrivelled pods to the same relative degree (13 to 15 percent) in all three cultivars, and, for cv. Chilean, premature pod abortion was estimated to be 22.6 percent. Subsequent experiments are needed to quantify the (1) relative contributions of host preference (cv. Precoz and accession LC800028 are exotic introductions); (2) stage of phenological development when plants were exposed to the bugs (the two exotic cultivars were slightly more advanced into the reproductive period, and may also have different blooming patterns and durations); and (3) effects on node production and so the proportion of nodes that become reproductive (by morphological modifications consequent upon bug damage to stem apices).

Estimated seed abortion due to *Lygus* was much more severe (11.0 to 19.17 percent) in the smaller-seeded accessions than in cv. Chilean (4.3 percent), as was the loss in individual seed dry weight due to chalky spot syndrome. Compared with individual seeds on control plants, those damaged by chalky spot were 26.1 percent lighter in cv. Chilean (and see table 3) and 40.7 to 54.0 percent lighter in the other two cultivars; however, as so often happens in grain legumes when reproductive load is reduced (Summerfield 1980a), the undamaged seeds on the poorer-yielding plants subjected to *Lygus* were atypically heavy (increases of 2.5 to 18 percent). Thus, the overall average individual seed dry weights of the populations of seeds from all Chilean and Precoz plants were remarkably invariant; however, the dramatic effects on seed size of chalky spot in LC800028 (with normal seeds only about one-third the weight of those produced by cv. Chilean) were not compensated by larger-than-normal undamaged seeds. These



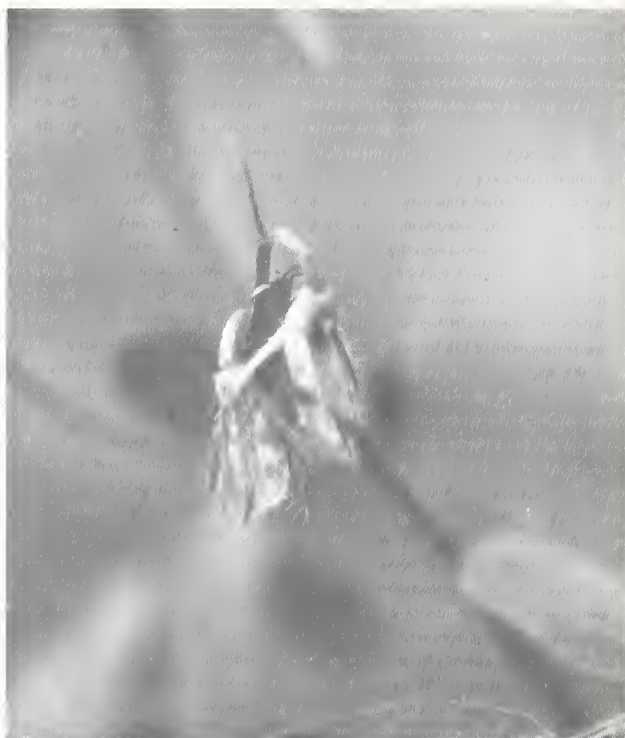


Figure 10.--Adults of *Lygus hesperus* as they were frequently observed on caged lentil plants grown in a glasshouse. Bugs seemed especially attracted to open flowers (top left), racemes on which petals had faded and collapsed but immature pods were not yet exposed (top right), and green pods (bottom). Stylet insertion has been observed in situations such as depicted in upper figures (top left and right).





Figure 11.--Localized exudates (left) and staining (right) of lentil pods on plants subjected to *Lygus* bug infestation in cages in a glasshouse.

Table 8.--Effects on seed yield and selected components of yield of caging *Lygus* bugs onto pot-grown plants in a glasshouse (mean values of 15 replicates  $\pm$  SE)

Attribute of seed yield measured or derived	Lentil cultivar					
	Control			With <i>Lygus</i>		
	Chilean	Precoz	LC800028	Chilean	Precoz	LC800028
No. pods plant <sup>-1</sup> .	102.8 $\pm$ 5.8	40.6 $\pm$ 1.7	62.4 $\pm$ 3.1	79.6 $\pm$ 5.3	40.9 $\pm$ 2.5	59.9 $\pm$ 3.5
No. shrivelled pods plant <sup>-1</sup> .	24.3 $\pm$ 3.8	3.7 $\pm$ .8	4.5 $\pm$ .9	29.4 $\pm$ 4.6	9.9 $\pm$ 1.1	12.1 $\pm$ 2.1
Percent shrivelled pods plant <sup>-1</sup> .	23.6	9.1	7.2	36.9	24.2	20.2
Increased damage (percent) due to <i>Lygus</i> .	-	-	-	+13.3	+15.1	+13.0
Estimated pod abortion (percent) due to <i>Lygus</i> damage.	-	-	-	+22.6	0	+4.0
No. seeds plant <sup>-1</sup> .	96.9 $\pm$ 4.0	49.7 $\pm$ 2.2	91.3 $\pm$ 3.7	71.3 $\pm$ 4.4	40.2 $\pm$ 3.9	77.7 $\pm$ 5.9

Table 8.--Effects on seed yield and selected components of yield of caging *Lygus* bugs onto pot-grown plants in a glasshouse (mean values of 15 replicates  $\pm$ SE)--Continued

Attribute of seed yield measured or derived	Lentil cultivar					
	Control			With <i>Lygus</i>		
	Chilean	Precoz	LC800028	Chilean	Precoz	LC800028
No. chalky spot seeds plant <sup>-1</sup> .	0	0	0	28.7 $\pm$ 2.8	6.6 $\pm$ 0.6	16.6 $\pm$ 1.3
Percent chalky spot seeds.	0	0	0	40.2	16.4	21.4
No. seeds pod <sup>-1</sup>	.94	1.22	1.46	.90	0.98	1.30
Estimated seed abortion (percent) due to <i>Lygus</i> damage.	-	-	-	+4.3	+19.7	+11.0
Mean seed dry weight mg:						
Chalky spot seeds	-	-	-	30.9	23.0	7.5
Undamaged seeds	41.7	38.8	16.3	49.2	42.4	16.7
Overall mean.	41.7	38.8	16.3	41.8	39.2	14.7
Estimated change in overall mean seed dry weight (percent) due to <i>Lygus</i> damage.	-	-	-	+ .24	+1.03	-9.8
Seed yield (g) plant <sup>-1</sup>	4.04 $\pm$ 1.16	1.93 $\pm$ .11	1.49 $\pm$ .09	2.98 $\pm$ .17	1.58 $\pm$ .17	1.14 $\pm$ .10
Loss in seed yield (percent) due to <i>Lygus</i> damage.	-	-	-	-26.2	-18.1	-23.5

observations are consistent with findings elsewhere; in general, smaller-seeded species suffering *Lygus* attacks seem likely to experience greater relative losses of individual seed dry matter and of economic yield than species genetically programmed to produce large seeds (for example, Flemion et al. 1949; Gupta et al. 1980).

Plants caged with *Lygus* bugs throughout reproductive growth yielded an average of 22.6 percent less seed dry weight than those grown in an identical cage but free of insects. Thus, in this forced feeding situation at least, not only the quality but also the quantity of economic yield was reduced. Of course, as we suggested in the Introduction, farmers may not be aware that their seed yields have been reduced, since shrivelled pods and lighter seeds are likely to be lost from the combine at harvest. Only by hand harvesting plots in

carefully designed field experiments will it be possible to relate these data on loss of economic yield in the artificial situation studied here to that in crops. Such studies merit priority in subsequent research programs--as we discuss later.

### Chalky Spot Damage, Cookability, and Taste

Average values of flavor characteristics of cooked lentil samples, containing either 0, 10, or 20 percent chalky spot seeds as assessed by sensory panelists, are presented in table 9. Descriptive terms used by the panelists

Table 9.--Mean values of sensory characteristics (taste panel), shear force (kilograms), hunter-color and drained weight (grams) of cooked lentil samples comprising various proportions of chalky spot seeds

Attribute of cooked sample	Sample composition (undamaged:damaged seeds (percent) by weight)		
	100:0	90:10	80:20
Sensory characteristics <sup>1</sup> :			
Flavor	3.4	3.8	3.5
Texture	3.6	3.9	3.7
Appearance	3.8	2.9	3.3
Preference score	2.68	2.60	3.10
Shear force value (kg) <sup>2</sup>	36.1	35.7	34.9
Hunter-color:			
Rd (lightness)	8.7	7.9	8.2
a (redness)	2.0	2.5	2.2
b (yellowness)	14.5	13.5	14.0
Drained weight (g) <sup>3</sup> :			
Cooked lentils	294	293	300
Cooking medium	72	84	80

<sup>1</sup>Mean values of 24 panelists asked to assess the degree of difference from a control sample (100:0) on a 9-point scale (1 = no difference to 9 = extreme difference).

<sup>2</sup>Mean values of 6 estimates.

<sup>3</sup>Mean values of weights of samples prepared for panels 2 and 3.

indicated that samples containing chalky spot seeds were "slightly less sweet" and "a little more bland" than the undamaged control samples. In texture, the samples containing damaged seeds had "slightly tougher seedcoats", which is perhaps not surprising since chalky spot seeds are known to have thicker testas (table 3). Shear press values were slightly smaller for the inferior samples,

presumably indicating an increased tendency to become mushy during processing. (Note, in table 9, the increased weight of cooking medium for these samples, reflecting, perhaps, greater losses of seed integrity during baking.)

Overall, although lentil seed lots comprising either 10 or 20 percent chalky spot seeds (by weight) were slightly inferior in sensory characteristics and texture to the undamaged samples used in this pilot study (table 9), differences were not significant statistically. Nevertheless, the presence of chalky spot seed detracted from the visual and sensory appeal of the processed product and, if present in proportions greater than 20 percent by weight, the negative tendencies described above may become correspondingly greater. On the other hand, seed lots containing, say, 10 percent, or perhaps even 20 percent chalky spot seeds should not be automatically downgraded on the basis of these criteria alone.

## FUTURE RESEARCH NEEDS AND OBJECTIVES: RETROSPECT, CONCLUSIONS, AND PROSPECT

After a seed is formed on the mother plant, there is an initial period of immaturity during which it reaches its full potential; the seed then goes through a series of deteriorative changes, beginning on the mother plant and continuing through harvest, drying, processing, and storage (Roberts and Ellis 1980). Deterioration proceeds inexorably at a rate dependent on temperature and water content, but can be accelerated by other factors. Then again, seed formation may be prevented completely if flowers are lost; immature seeds may abort; development may be arrested during the time course of embryogenesis (Dure 1975); or mature seeds may have "abnormal" characteristics, which result in failure to meet certain standards of quality demanded by growers themselves, processors, seed merchants, or consumers. Chalky spot syndrome of lentil seeds undoubtedly reduces the quality of a seed lot--as any grower whose crops suffer the problem will attest--but are economic yields also reduced? What has been achieved hitherto and what remains to be done to remove this constraint to improve lentil production from the Palouse area?

Several species of insects feed on seeds as they mature in the field. Feeding activities may stimulate abortion; they may partially or completely destroy the seed, kill embryos, or variously scar, spot, pit, sting, or discolor testa and/or cotyledons. Although *Lygus* spp. have probably been the subject of most research--these omnivorous feeders attack more than 50 species of economic importance as well as many weeds and grasses (Knight 1941)--other pests produce similar feeding damage to seeds. For example, *Adelphocoris* spp., another member of the Miridae, several stinkbugs of the family Pentatomidae (for example, *Chlorochroa sayi*, *Euschistus variolarius*, and *E. impictiventris*), and *Thyanta custator* have been found to cause similar symptoms to those that result from feeding *Lygus* bugs on beans, alfalfa, sugarbeet, and cotton (Flemion et al. 1949). Then again, "physiological spotting" of pea seeds (which is usually accompanied by cavitation (hollow heart) or the adaxial surfaces of cotyledons) has been related to nutritional disorders (Zaunmeyer 1962), and the formation of embryoless seeds in cereals, castor beans, and ginko is thought to be caused by genetic factors, pollen defects, nutrition, or other causes (Baker 1972). From this spectrum of possibilities that might conceivably explain the malformation of lentil seeds known to growers as chalky spot syndrome, we favored a hypo-



thesis that *Lygus* bugs feeding on immature reproductive structures were responsible for the damage. The fact that *Lygus* spp. have not to our knowledge (which includes a survey of more than 2,000 citations in the world literature on lentils) been reported as either a major or minor pest of the crop did not daunt us.

In seeking mutually supporting evidence to support or refute our hypothesis, we have called upon the respective skills of a diverse range of specialists (see Acknowledgments). This approach emphasizes yet again (and see Summerfield et al. 1982) that problems related to the improvement and stability of seed yields from lentil crops are best approached by a multidisciplinary, collaborative effort. By including such a wide range of topics, however, several recommendations for subsequent research might now be advanced. Notwithstanding, we have been guided by estimates of the relative costs and timeliness of different research and development projects, and benefits to the industry that may accrue from the successful outcome and adoption of research and development findings (see, for example, Wingate-Hill and Davis 1976), in the recommendations that follow.

Although we have attributed some significance to the apparent prevalence of chalky spot syndrome in certain areas in the Palouse (fig. 1), different graders of seed lots may not be adopting equally stringent criteria in their assessments of lentil seed quality. Studies to assess this possibility and to establish acceptable standards and consistency of interpretation are needed to more reliably estimate the prevalence of chalky spot syndrome on Palouse lentils (and see Hawkins 1980).

Despite the wealth of evidence presented here which implicates *Lygus* bugs as the cause of chalky spot syndrome, we are still ignorant of the stage during crop life when most plants in a stand are susceptible to damage (other than the fact that damage must occur after the onset of blooming), the time during embryogenesis when seeds are likely to suffer most from feeding activity, and the species and stage during the life cycle of bugs most injurious to the crop. Appropriate experiments in glasshouses may circumvent much wasted time and effort in the field by pinpointing for various cultivars (say, Chilean, Redchief, and Brewer) the stage of plant development when damage is most serious and the species and age of bugs that are most injurious, and by quantifying the relationships between pest density and quality and quantity of yield. It would be prudent, of course, to complement such experiments by a judicious use of cages and known pest introductions in crop situations. Only when these data become available will it be possible to time sampling activities designed to monitor pest populations in the field with any reasonable certainty of return.

Research on insecticides must obviously be included in any attempts to develop a pest management package, but to identify the most effective pesticide is not the primary objective (van Emden 1980). For pest management on a sound ecological background, experiments are needed that are likely to generate ecological data on (a) forecasting attack (the numbers of the pest reaching the crop, from whence they came, and the periods of time over which such arrival occurs); (b) estimating economic thresholds (to quantify the relationship between pest density and yield); (c) monitoring populations (common sampling techniques such as sweeping may well be unsuitable for grain legume crops; van Emden 1978); and (d) evaluating the impact of control packages (see the cogent discussions by van Emden 1978, 1980). Empirical studies to evaluate the potency



of alternative insecticide formations (for example, Hagel 1978) may be useful in the short term, so that appropriate compounds and rates of application can be registered and developed and then used legally by growers. In the longer term, these studies must be integrated into the pest management package<sup>7</sup> that must include weed management as a major component (Fye 1980). Indeed, alternate hosts, migration of adults, and prolonged hatching of nymphs (which may differ from adults in their response to insecticides; Smith and Michelbacher 1946) will make any "stiletto" pest control method (the one-for-one matching of pests and controls; van Emden 1980) rather difficult (Flemion et al. 1949).

Until sound field data become available, growers might be wise to avoid disking weeds along ditchbanks, roadsides, and in wasteland corners of irrigation pivots (and to use herbicides instead) and to sow lentil and Brassica crops well apart.

In describing our research on chalky spot syndrome in lentils--advocating feeding activities of *Lygus* bugs as the most likely cause of damage and suggesting what needs to be done in future research--we have been careful not to offer either a scenario or panacea. Research in the field will undoubtedly contribute significantly to practical and economically attractive strategies for minimizing the incidence of chalky spot syndrome in future lentil crops--not only by chemicals but also by cultural practices appropriate for maximum biological control.

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<sup>7</sup> Host plant resistance in combination with insecticide applications and natural *Lygus* enemies for optimum control (Gupta et al. 1980; van Emden 1980).

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